All required amendments to the specification have been made to put it into proper form. The Examiner's careful review of the specification is appreciated.

The claims have been amended in several respects as well. Such amendments will be discussed in more detail below, but a summary is presented here.

Claims 1, 12, 19, 22 have been amended to recite the amount of each primer being within the range of from about 0.1 to about 2 μmolar. Support for this is found on page 19 (lines 20-23) of the specification. These claims have also been amended to recite at least 10 units/10 μl of solution of the thermostable DNA polymerase. Support for this amendment is on page 19 (lines 14-16). Dependent Claims 2, 16 and 35 have also been amended to delete the broader scope of primer and DNA polymerase amounts to be consistent with the amended independent claims.

Claims 9, 15, 32, 34 and 38 have been amended to identify SEQ ID NO's as requested in the Office Action.

Claim 11 has been amended to correct an error in dependency. Claims 15, 27, and 30-35 have also been amended to change dependency in view of cancellation of Claims 14, 21, 28 and 29.

Kit Claims 12 and 19 have been amended to include the capture reagents of cancelled Claims 14 and 21. Claim 19 has also been amended to indicate that the third and fourth primers are present in a separate aqueous composition, as described on page 6 (lines 17-35) where each listed component of the kit, a), b) and c), is described as <u>in separate packaging</u>.

Method Claim 22 has been amended to indicate that in each PCR cycle, priming and primer extension is carried out at the same temperature within the range of from about 62 to about 75°C, as described on page 22 (lines 9-11) and in cancelled Claim 28. Each cycle is carried out within 120 seconds, as taught on page 22 (lines 1-3). This claim also now recites the capture of

the amplified target strands using the capture reagents of cancelled Claim 29.

Rejection Under 35 U.S.C. 112, Second Paragraph

Claims 1-8 and 10-38 have been rejected as being indefinite for failing to recite specific oligonucleotide sequences. As far as it applies to claims presently in this application, this rejection is respectfully traversed.

The Office Action objects to the functional nature of the recitation of oligonucleotides, and supports it arguments by reference to the treatment of chemical compound inventions by the P.T.O.B.P.I. in *Ex parte D.*

Granted that the Board indicates in the noted case that DNA sequences are complex chemical compounds. However, this case is irrelevant to the issue at hand. It related to a prior art rejection of a compound *per se*, not to aqueous soluions, test kits and assay methods, as in the present application. In the only compound claim of this application, Claim 38, Applicants have recited the nucleotide sequences claimed.

Similar to other cases involving "chemical compounds", Applicants are allowed by the Statute to recite the use of chemical compounds in functional terms, i.e. by what the primers and probes do, not by their structure, *In re Fuetterer* 138 U.S.P.Q. 217 (C.C.P.A., 1963). This case remains good law and has been followed for decades by the Board and C.A.F.C. The metes and bounds of Applicants claimed invention are well within the routine experimentation of a skilled artisan because Applicants have provided sufficient teaching in their specification as to how to choose appropriate primers and probes (pages 11-16). Other knowledge in the art would readily be available also to help one to adequately choose the appropriate sequences to fall within the scope of the claims. Conversely, a skilled artisan would know how to design around the invention because the metes and bounds are clearly defined in the claim language and specification. As is well known, the claims must not be

read in a vacuum, but in light of the specification and what is well known in the art, and thereby given the broadest interpretation possible, *In re Marosi et al* 218 U.S.P.Q. 289, at 292 (C.A.F.C., 1983). Therefore, the objection to the functional definition of the oligonucleotides in the claims is in error.

Claim 11 has been amended to correct the dependency.

Claim 22 has been amended to recite "the *additional* PCR reagents" used in the amplification to provide antecedent basis for later claims.

Claims 31 and 37 have been rejected as indefinite because of alleged lack of clarity as to how the capture reagents are disposed on the substrate. Applicants disagree with this characterization of this claim language. They have described in the specification (page 28, lines 5-25) how such reagents are disposed on the substrates. In addition, such information is also provided in published literature (e.g. U.S. Patent 5,173,260). Because the claims are to be read in view of the teaching in the specification and what is known in the art, *In re Marosi, supra,* Applicants' have provided a sufficiently clear indication of the metes and bounds of the invention.

Similarly, the rejection of Claim 37 is in error. Diagnostic elements are well understood from the art (e.g., U.S. Patent 5,173,260 and WO 92/16659) and the teaching in Applicants' specification (page 28). One skilled in the art would understand such a device to be an article on which several capture reagents are disposed, either by coating, chemical attachment or other means. The definition of a "water-insoluble, heat or ultrasonic sealable support" is likewise understood by a skilled artisan, who would be a scientist who designs assay devices using oligonucleotide capture reagents. Many such scientists are working in both large and small companies in the United States.

Claim 38 has been amended to better define the recited oligonucleotide sequences.

All of the claims are now believed to meet the requirements of Section 112(2), and the rejection under that portion of the Statute should therefore be withdrawn.

Rejection Under 35 U.S.C. 112, Fourth Paragraph

Claim 19 has been rejected as directed to the same subject matter as Claim 12. This is not the case, but Claim 19 has been amended to make that distinction more clear. The third and fourth primers are in a separate aqueous solution from the first and second primers, as indicated on page 6 of the specification. Claim 12 is directed to a kit wherein all primers are in the same packaged solution whereas the kit of Claim 19 has two separately packaged solutions. This is now clear in the amended claim.

Rejection Under 35 C.F.R. 112, First Paragraph

The specification has been objected to and Claims 22-27 and 29-36 have been rejected as lacking sufficient enabling disclosure because the amplification cannot allegedly be carried out without specific conditions. With respect to the presently claimed invention, this rejection is respectfully traversed.

Claim 22 has been amended to incorporate the language of cancelled Claim 28 such that primer annealing and primer extension in each PCR cycle is carried out within a specific temperature range. This range is consistent with the Tm values of of the recited primers. Thus, simultaneous amplification of multiple target nucleic acids can be carried out using the combination of "matched" primers and probes recited in the claims. This amendment therefore obviates the rejection under Section 112(1).

Rejection Under 35 U.S.C. 102(b)

Claim 38 has been rejected as anticipated by EP 462,644 (Sutton et al). This rejection is respectfully traversed.

The Office Action alleges that SEQ ID NO:1, 2 and 4-8 are described in Sutton et al. At least some of these oligonucleotides are shown in the cited reference.

The Office Action is incorrect, however, in stating that such oligonucleotides are recited in Claim 38. As pointed out later in the Office Action, Claim 38 recites SEQ ID NO:3, and 16-26 (consistent with Applicants' page 30). Obviously, this does not include the sequences shown in Sutton et al. Thus, the subject matter of Claim 38 is novel and not anticipated by Sutton et al. The rejection under Section 102(b) is in error and should be withdrawn.

Rejection Under 35 U.S.C. 102(f)

Similarly, this rejection is in error because the sequences of Claim 38 are not disclosed in Sutton et al. Thus, there is no need to clarify inventorship of the novel sequences of Claim 38. They were invented by one or more of the Applicants listed on the present application. A so-called Katz-type declaration is unnecessary in this instance.

Rejection Under 35 U.S.C. 103

Claim 38 has been rejected as unpatentable over a publication by Brytting et al. This rejection is respectfully traversed for the following reasons.

Brytting et al describes on pages 129-130 several nested primers for amplification of human CMV DNA. These are not the same oligonucleotides recited in Claim 38.

The Office Action alleges that Applicants have admitted the availability of hCMV and pro-virus ev-1 DNA. Applicants are not certain of what statements the Examiner is referring to, but it is well known that some sequences of the hCMV DNA of certain strains are known, but there are still unknown sequences in various strains. There is no guarantee or reasonable predictability that any set of primers will be useful to efficiently amplify targets from various strains of the same virus. While it is true that designing primers for PCR is a routine feature of diagnostic assay development, it is not obvious to develop primers and probes with "matched" Tm's in order to amplify and detect several targets simultaneously using the same PCR conditions, as

Applicants have done. There is no teaching in Brytting et al that any particular primer set would be useful in the multiplexing method that Applicants have developed, and thus primers useful in such methods would not be obvious from the teaching of specific primers by Brytting et al.

It should be noted that in Claim 38, SEQ ID NO:3 and 16-18 are oligonucleotides directed to hCMV DNA sequences, SEQ ID NO:19, 20 and 25 are oligonucleotides directed to pro-virus en-1 DNA, and SEQ ID NO:21-24 and 26 are directed to synthetically prepared targets which have no relation to either hCMV or pro-virus en-1 DNA. Thus, while Brytting et al may appear to have some pertinence to SEQ ID NO:3 and 16-18, Brytting et al is not relevant art under the Statute for the remaining oligonucleotides.

For these reasons, the rejection of Claim 38 should be withdrawn.

Claims 1-37 have been rejected as unpatentable over the combined teaching of Nedjar et al, Brytting et al, Gibbs et al and Findlay et al (WO 90/08840). As far as it applies to claims presently in this rejection, this rejection is respectfully traversed.

As pointed out above, the claims have been amended in several respects which are important to consider in view of the cited art. All independent claims now recite a limited amount of each primer and high amount of DNA polymerase, which amounts Applicants submit are important for rapid, efficient and simultaneous amplification in their multiplexing invention. Thus, while the matching Tm's are important, as stated in the specification, the amount of primer and DNA polymerase are also very important in order to achieve the desired simultaneous amplification in a rapid fashion (each cycle less than 120 seconds).

Moreover, method Claim 22 now recites that each amplification cycle is carried out using the same temperature for primer annealing and primer extension, which temperature is in a narrow range to suit the

matched primers used in the method. Thus, the claim more clearly distinguishes the method from routine PCR where one temperature is used for primer annealing and another temperature is used for primer extension. Applicants have found that certain sets of primers, when "matched" as recited in the claims, can be advantageously used to amplify multiple targets simultaneously, as opposed to sequentially, in the presence of specific amounts of primers and DNA polymerase. Applicants' so-called "two-temperature" PCR (one temperature for denaturation, and the second for primer annealing and extension) requires the "matched" primers for multiple target amplification, and such a method is not suggested by the combined art cited in the Office Action which teaches "three-temperature" PCR (separate temperatures for denaturation, primer annealing and primer extension).

The rapid cycle (less than 120 seconds) for Applicants' method is highly desirable, and achievable for multiple targets only with the use of the recited amounts of primer and DNA polymerase and the use of "matched" primers which are thereby very efficient under the stringent PCR conditions required for rapidity and efficiency. None of the cited art, alone or in combination, suggests this critical combination of features needed for rapid and efficient amplification of multiple target nucleic acids.

Nedjar et al relates to coamplification of HCV and HIV-I DNA. It does not relate to detection of hCMV DNA. Thus, the characterization of the Office Action on page 6 that the "DNA sequence of human CMV is taught in the prior art which has been used by both Nedjar et al and Brytting et al to design primers..." is incorrect. It is clear from a careful reading of Nedjar et al that it teaches the use of "nested" PCR (see page 299) for detection of two target nucleic acids. As one skilled in the art understands, "nested" PCR requires the use of different primer sets in sequence, not simultaneously. Applicants' method utilizes multiple "matched" primer sets simultaneously in the same reaction solution. This

is clear from reading the language of the method and composition claims and the teaching of Applicants' specification. The "nested" PCR method of Nedjar et al would certainly not suggest Applicants' simultaneous amplification of multiple targets using specially "matched" sets of primers.

Moreover, Nedjar et al teaches a very slow PCR procedure (at least 8 minutes for each cycle, see page 299) compared to Applicants' very rapid (two minutes or less for each cycle) method. Further, Nedjar et al teaches two different primer annealing and extension temperatures (37°C and 72°C) which necessitate very long cycle times since lengthy times are needed for moving from one temperature to another ("ramping" times). Applicants' claims recite a single temperature for both steps. Still further, Nedjar et al teaches the use of merely 2.5 units of DNA polymerase per 100 µl of solution. This is contrasted with Applicants' claims reciting at least 10 units/100 µl.

Clearly then, Nedjar et al is severely deficient as a primary reference in teaching the presently claimed invention. The question is whether the secondary references provide the missing teaching or any motivation to put the missing pieces together. Applicants submit that they do not.

Brytting et al fails to provide any teaching to overcome the deficiencies of Nedjar et al. Admittedly, it relates to amplification of hCMV DNA as opposed to HCV, but it also teaches "nested" PCR (see pages 129-131) using nested primer pairs. Moreover, different temperatures were used in amplification for primer annealing and primer extension (page 131, first paragraph). Contrary to that teaching, Applicants claim PCR using multiple primer sets simultaneously ("non-nested" PCR) and a single temperature for primer annealing and extension. Moreover, Brytting et al teaches the use of only 1 unit DNA polymerase/50 µl of solution (2 units/100 µl). Admittedly, the Brytting et al cycle was shorter than most (90 seconds), but there is

no suggestion that multiple targets can be amplified this quickly in the same reaction mixture. Rather, the "fast" cycles are used in "nested" PCR where primer sets are used in different cycles. Applicants' different invention using "matched" primers, is not suggested by Brytting et al and Nedjar et al together.

Findlay et al admittedly teaches various diagnostic elements or articles having capture probes disposed thereon. However, it fails to overcome the deficiencies noted above in Brytting et al and Nedjar et al. It should be noted also that the existence of Findlay et al is supportive of Applicants' earlier argument that the Section 112(2) rejection of the element claim is in error since the basic structure of such articles is well known. Applicants, however, have provided a novel and patentable improvement by putting "matched" probes on such articles for use in the rapid PCR method recited in Claim 22.

Gibbs et al is cited for its alleged motivation to simultaneously detect several target nucleic acids using multiple primer sets. This is admittedly more pertinent art that any of Nedjar et al, Brytting et al and Findlay et al. However, it fails to teach or suggest Applicants' claimed invention, by failing to provide the teaching missing from the other cited publications.

Gibbs et al is a detailed analysis of research for finding optimum primer sets for various sequences in the hypoxanthine phosphoribosyltransferase gene. The reference suggests that the desired primers have a critical amount of GC content.

Applicants' claimed method is distinguishable thereover. Applicants method is a "rapid" PCR procedure for amplification of multiple targets wherein each cycle is 120 seconds or less. In order to accomplish this, Applicants require "matched" primers, the same concentration of each primer and a high amount of DNA polymerase.

Gibbs et al describes a process for finding out the optimum amounts of primers needed for detecting

multiple sequences on the same gene (see paragraph bridging pages 236-237). Because the multiple targets were detected using gels, they were necessarily of different lengths, and thus different amounts of primers were also necessary. Thus, Gibbs et al teaches that the amounts of each primer set had to be adjusted to compensate for uneven signal strength when they were used in the same reaction mixture (see page 238, paragraph bridging the columns). The amounts of primers ranged from 10 pmol to 25 pmol which is several orders of magnitude less than Applicants' range of from about 0.1 to about 2 μmolar.

In addition, Gibbs et al teaches multiple amplification using only 8 units DNA polymerase/100 μl, and separate temperatures for primer annealing and primer extension (see paragraph bridging pages 236-7). Each cycle was at least 3 minutes. Detection of the multiple analytes in Gibbs et al was achieved by using conventional gels, not "matched" capture probes as in the present invention.

The PCR of Gibbs et al does not teach Applicants' claimed composition, kit and method whereby considerably more of each primer is used in the reaction mixture. Moreover, Applicants' use of more rapid cycles and the same temperature for primer annealing and primer extension is not suggested by Gibbs et al. Thus, Gibbs et al fails to teach the combined features of Applicants' method which enable rapid and efficient amplification and detection of multiple targets by means of capture probes.

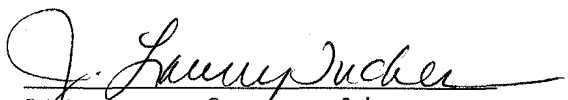
Gibbs et el merely suggests that one can detect multiple targets if one optimizes the amount of each primer used and the targets are of different lengths. Applicants's method is not under such constraints. The amount of primer need not be optimized within the recited range, and the targets can be of any length because capture is not dependent upon resolution on gels. Moreover, Applicants have a more rapid process and have avoided the need for multiple temperatures in each PCR cycle. Only Applicants' combination of "matched"

primers, and primer and DNA polymerase amounts make this possible.

The Office Action has failed to present a *prima facie* case for obviousness. While some pieces of the claimed invention are shown in the art, the combination of critical features is lacking in actual description and suggestion. No nexus between the cited art and the claimed invention has been pointed out, and it is merely the Examiner's opinion that one skilled in the art would be able, with reasonable predictability, achieve what Applicants have done, particularly in view of the amendments to the claims. Thus, the rejection should be withdrawn.

All of the issues presented in the Office Action have been addressed, and it believed that the present application in is condition for allowance. If the Examiner has any questions or further amendments to suggest, he is encouraged to call the undersigned so resolution of all issues, and allowance of this application, can be expedited.

Respectfully submitted,



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